Lutimaribacter saemankumensis gen. nov., sp. nov., isolated from a tidal flat of the Yellow Sea

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A Gram-negative, non-motile, rod-shaped bacterial strain, designated SMK-117^T, belonging to the *Alphaproteobacteria*, was isolated from a tidal flat of the Yellow Sea, Korea, and was subjected to a polyphasic taxonomic study. Strain SMK-117^T grew optimally at pH 7.0–8.0 and 30 °C in the presence of 2% (w/v) NaCl. Neighbour-joining and maximum-likelihood phylogenetic trees based on 16S rRNA gene sequences showed that strain SMK-117^T clustered with *Maritimibacter alkaliphilus* HTCC2654^T, with which it exhibited a sequence similarity of 95.3%. Strain SMK-117^T contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c and 11-methyl C_{18:1}ω7c as the major fatty acids. The major polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and two unidentified phospholipids. The DNA G+C content was 63.5 mol%. Strain SMK-117^T was differentiated from members of the genera *Maritimibacter* and *Oceanicola* on the basis of differences in the fatty acid and polar lipid profiles. The phenotypic, chemotaxonomic and phylogenetic data indicated that strain SMK-117^T represents a novel genus and species, for which the name *Lutimaribacter saemankumensis* gen. nov., sp. nov. is proposed. The type strain of *Lutimaribacter saemankumensis* is SMK-117^T (=KCTC 22244^T =CCUG 55760^T).

A tidal flat is a part of a coast that is covered with seawater during the flood tide and left covered with mud or slime at ebb tide. In recent years, bacteria belonging to the *Proteobacteria* have been isolated from a variety of tidal flats on the west coast of the Korean peninsula (Yi *et al.*, 2003, 2004; Yoon *et al.*, 2004, 2005, 2007b; Baik *et al.*, 2005; Jung *et al.*, 2006, 2007). In this study, we describe a bacterial strain, designated SMK-117^T, isolated from a tidal flat sediment at Saemankum (Yellow Sea, Korea). The aim of the present work was to determine the exact taxonomic position of strain SMK-117^T by using a polyphasic characterization that included determination of its phenotypic and chemotaxonomic properties and a detailed phylogenetic investigation based on its 16S rRNA gene sequence.

Strain SMK-117^T was isolated from a tidal flat sediment by means of the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Difco). *Maritimibacter alkaliphilus* KCCM 42376^T was obtained from the Korean Culture Center of Microorganisms (Seoul, Republic of Korea), *Oceanicola nanhaiensis* LMG 23508^T and *Oceanicola marinus* LMG 23705^T were obtained from the

Laboratorium voor Microbiologie (Universiteit Gent, Ghent, Belgium) and *Oceanicola batsensis* KCTC 12145^T and *Oceanicola granulosus* KCTC 12143^T were obtained from the Korean Collection for Type Cultures (Taejeon, Republic of Korea). The morphological, physiological and biochemical characteristics of strain SMK-117^T were investigated using routine cultivation on MA at 30 °C. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). Flagellation was investigated by using transmission electron microscopy on cells (from an exponentially growing culture) negatively stained with 1% (w/v) phosphotungstic acid (see Supplementary Fig. S1, available in IJSEM Online). Grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1%, w/v), both of which had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was omitted. Growth at various NaCl concentrations (0.5%, w/v, and 1.0–13.0%, w/v, in increments of 1.0%) was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth at various temperatures (4, 10, 15, 20, 25, 28, 44, 45, 46 and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SMK-117^T is EU336981.

A transmission electron micrograph of cells of strain SMK-117^T from exponentially growing culture is available as supplementary material with the online version of this paper.

47 °C and from 30 to 40 °C in 1 °C increments) was measured on MA. The pH range for growth was determined in MB that was adjusted to various pH values (pH 4.5–9.5, in increments of 0.5 pH units). Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, starch and Tweens 20, 40, 60 and 80, hypoxanthine, tyrosine and xanthine was tested on MA, using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were investigated as described previously (Lányi, 1987) but with the modification that artificial seawater was used for the preparation of media. The artificial seawater contained (l^{-1} distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2 \cdot 6H_2O$, 5.94 g $MgSO_4 \cdot 7H_2O$ and 1.3 g $CaCl_2 \cdot 2H_2O$ (Bruns *et al.*, 2001). H_2S production was tested as described previously (Bruns *et al.*, 2001). Susceptibility to antibiotics was investigated on MA plates by using antibiotic discs containing the following: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg) and tetracycline (30 µg). Acid production from carbohydrates was tested as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Baumann & Baumann (1981), using supplementation with 0.01 % (w/v) yeast extract. The carbon sources were added at a concentration of 0.2 % (w/v) after sterilization by filtration. Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux). For spectral analysis of *in vivo* pigment absorption, strain SMK-117^T was cultivated aerobically in the dark at 30 °C in MB. The culture was washed twice using centrifugation with a MOPS buffer (MOPS/NaOH, 0.01 M; KCl, 0.1 M; $MgCl_2$, 0.001 M; pH 7.5) and disrupted by means of sonication (450 sonifier; Branson). After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer.

Cell biomass of strain SMK-117^T for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown in MB at 30 °C. Cell biomass of *M. alkaliphilus* KCCM 42376^T for polar lipid analysis was obtained from cultures grown in MB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination by RNA. The 16S rRNA gene was amplified using a PCR with two universal primers (5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAGCC-3') as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC. Polar lipids were extracted according to the procedures described by Minnikin *et al.*

(1984) and were identified by means of two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The presence of phosphatidylcholine was tested by spraying Dragendorff's reagent (Sigma). For cellular fatty acid analysis, cell mass of strain SMK-117^T, *M. alkaliphilus* KCCM 42376^T, *O. nanhaiensis* LMG 23508^T, *O. marinus* LMG 23705^T, *O. batsensis* KCTC 12145^T and *O. granulosus* KCTC 12143^T was harvested from MA plates after cultivation for 3 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by using the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed using nuclease P1 (Sigma) and the resultant nucleotides were analysed by means of reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain SMK-117^T are given in the species description (see below) or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain SMK-117^T determined in this study comprised 1420 nt. After gaps at the 5' and 3' ends of the alignment had been omitted, the remaining 1383 positions were used in the phylogenetic analysis. In the phylogenetic tree based on the neighbour-joining algorithm, strain SMK-117^T joined the lineage comprising *M. alkaliphilus* at a bootstrap support value of 72.2 % (Fig. 1). This topology was also found in the tree constructed using the maximum-likelihood algorithm (Fig. 1). Strain SMK-117^T exhibited 16S rRNA gene sequence similarity of 95.3 % with respect to *M. alkaliphilus* HTCC2654^T, 94.3–95.5 % with respect to the type strains of *Oceanicola* species and <94.6 % with respect to the other species used in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain SMK-117^T was Q-10, at a peak area ratio of approximately 94 %. The cellular fatty acid profile of strain SMK-117^T is shown in Table 2, together with those of the type strains of *M. alkaliphilus* and *Oceanicola* species analysed in this study. The major fatty acids in strain SMK-117^T were $C_{18:1\omega7c}$ (68.7 %) and 11-methyl $C_{18:1\omega7c}$ (12.3 %) and the major polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and two unidentified phospholipids. The DNA G+C content of strain SMK-117^T was 63.5 mol%.

The phylogenetic trees constructed using the neighbour-joining and maximum-likelihood algorithms indicated that strain SMK-117^T clusters with the type strain of *M. alkaliphilus* (Fig. 1). However, strain SMK-117^T was distinguishable from *M. alkaliphilus* KCCM 42376^T on the basis of differences in the cellular fatty acid and polar lipid profiles (Table 2). The fatty acids $C_{16:0}$ and $C_{16:0}$ 2-OH were minor components in strain SMK-117^T, whereas they were major components in *M. alkaliphilus* KCCM 42376^T (Table 2). An unidentified aminolipid was detected

Table 1. Differential phenotypic characteristics of strain SMK-117^T and members of some phylogenetically related genera

Taxa: 1, strain SMK-117^T; 2, *Maritimibacter* (data from Lee *et al.*, 2007); 3, *Oceanicola* (Cho & Giovannoni, 2004; Gu *et al.*, 2007; Lin *et al.*, 2007); 4, *Roseivivax* (Suzuki *et al.*, 1999); 5, *Salipiger* (Martínez-Cánovas *et al.*, 2004); 6, *Donghicola* (Yoon *et al.*, 2007a); 7, *Roseisalinus* (Labrenz *et al.*, 2005). All taxa are positive for oxidase. +, Positive; –, negative; v, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Cell shape	Rod	Short rod	Short rod	Rod	Rod	Coccus or rod	Rod
Motility	–	–	–	+	–	–	+
Pigmentation	Pale yellow	Beige	Yellow	Pink	–	–	Red
Bacteriochlorophyll <i>a</i>	–	–	–	+	–	–	+
Growth at/with:							
4 °C	–	–	v ^{a*}	ND	–	–	+
40 °C	+	–	v ^a	ND	+	+	–
pH 10–12	–	+	–	ND	–	ND	–
10 % (w/v) NaCl	+	–	v ^b	+	+	+	ND
Catalase	+	+	v ^c	+	+	+	+
Nitrate reduction	–	–	v ^d	v	–	+	+
Acid production from glucose	–	+	– or ND†	+	–	+	–
Hydrolysis of:							
Aesculin	–	–	v ^e	ND	–	–	ND
Gelatin	–	–	– or ND†	v	–	–	–
Alkaline phosphatase (API ZYM)	–	+	+‡	ND	ND	+	ND
Polar lipids§	PC, PG, PE, AL, PLs	PC, PG, PE, NP	ND	ND	ND	ND	DPG, PG, PC
DNA G + C content (mol%)	63.5	61.7	64.7–71.5	59.7–64.4	64.5	59.7	67

*Variable results reported as follows: *a*, all species positive except *O. nanhaiensis*; *b*, all species positive except *O. marinus*; *c*, all species positive except *O. granulosis*; *d*, all species negative except *O. nanhaiensis* (weakly positive); *e*, positive for *O. granulosis* and *O. marinus*, negative for *O. batsensis*, no data available for *O. nanhaiensis*.

†No data available for *O. marinus*.

‡Data available for *O. marinus* only.

§AL, Unidentified aminolipid; DPG, diphosphatidylglycerol; NP, unidentified non-polar lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid.

in strain SMK-117^T, but it was not detected in *M. alkaliphilus* (Lee *et al.*, 2007). An unidentified non-polar lipid was present as a major constituent in *M. alkaliphilus*, whereas it was absent in strain SMK-117^T (Lee *et al.*, 2007); the same polar lipid profile for *M. alkaliphilus* KCCM 42376^T was also observed in this study. Strain SMK-117^T was distinguished from type strains of *Oceanicola* species in terms of some fatty acids, i.e. C_{16:0}, 11-methyl C_{18:1}ω7c, C_{19:0} cyclo ω8c or C_{18:1}ω7c (Table 2). Strain SMK-117^T is also distinguishable from some phylogenetically related genera through differences in some phenotypic properties (listed in Table 1). On the basis of phylogenetic data and the differential chemotaxonomic and phenotypic properties, strain SMK-117^T represents a novel genus and species within the *Alphaproteobacteria*, for which the name *Lutimaribacter saemankumensis* gen. nov., sp. nov. is proposed.

Description of *Lutimaribacter* gen. nov.

Lutimaribacter (Lu'ti.ma.ri.bac'ter. L. n. *lutum* mud; L. gen. n. *maris* of the sea; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Lutimaribacter* rod from sea mud).

Cells are Gram-negative, non-flagellated rods. Catalase- and oxidase-positive. Facultatively anaerobic. The predominant ubiquinone is Q-10. The major fatty acids (constituting >10 % of total fatty acids) are C_{18:1}ω7c and 11-methyl C_{18:1}ω7c. The type species is *Lutimaribacter saemankumensis*.

Description of *Lutimaribacter saemankumensis* sp. nov.

Lutimaribacter saemankumensis (sae.man.kum.en'sis. N.L. masc. adj. *saemankumensis* from Saemankum, the location on the Yellow Sea in Korea where the type strain was isolated).

Has the following characteristics in addition to those described for the genus. Cells are 0.4–0.8 µm wide and 1.0 to at least 10.0 µm long and non-motile. Colonies on MA are circular, slightly convex, smooth, glistening, pale yellow in colour and 1.0–1.5 mm in diameter after 7 days incubation at 30 °C. Growth occurs at 10 (weakly) and 45 °C, but not at 4 or 46 °C. The optimal pH for growth is

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain SMK-117^T and some related taxa. Bootstrap percentages (based on 1000 replications) >50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. The sequence of *Stappia stellulata* IAM 12621^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

between 7.0 and 8.0; growth occurs at pH 5.5, but not at pH 5.0. Growth occurs in the presence of 11% (w/v) NaCl, but not in the absence of NaCl or in the presence of >12% (w/v) NaCl. Anaerobic growth occurs on MA and on MA supplemented with nitrate. Tweens 20, 40, 60 and 80 are hydrolysed, but casein, hypoxanthine, xanthine, starch and L-tyrosine are not. H₂S is not produced. In assays with the API ZYM system, esterase (C4), esterase lipase (C8) and leucine arylamidase are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. D-Mannose is utilized and citrate is weakly utilized, but L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, sucrose, trehalose, D-xylose, acetate, benzoate, formate, L-malate, pyruvate, salicin, succinate and L-glutamate are not utilized. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, oleandomycin, penicillin G, polymyxin B and streptomycin, but not to lincomycin or tetracycline. The

Table 2. Cellular fatty acid compositions (%) of strain SMK-117^T, *M. alkaliphilus* KCCM 42376^T and the type strains of *Oceanicola* species

Strains: 1, SMK-117^T; 2, *M. alkaliphilus* KCCM 42376^T; 3, *O. nanhaiensis* LMG 23508^T; 4, *O. batsensis* KCTC 12145^T; 5, *O. granulosus* KCTC 12143^T; 6, *O. marinus* LMG 23705^T. Data were obtained from this study. Fatty acids that represented <0.5% in all strains have been omitted. –, Not detected.

Fatty acid	1	2	3	4	5	6
Straight-chain fatty acids						
C _{12:0}	–	–	0.1	1.5	–	–
C _{14:0}	–	0.3	–	1.0	–	0.5
C _{15:0}	1.0	0.8	0.2	1.8	–	–
C _{16:0}	2.8	13.8	7.8	14.7	13.2	26.0
C _{17:0}	4.9	1.4	0.6	5.7	0.7	0.4
C _{18:0}	0.9	0.8	1.1	2.4	0.9	1.7
Unsaturated fatty acids						
C _{17:1} ω6c	1.1	–	–	–	–	–
C _{17:1} ω8c	1.1	–	0.2	0.7	–	–
C _{18:1} ω7c	68.7	39.8	46.8	42.3	71.9	17.5
Hydroxy fatty acids						
C _{10:0} 3-OH	1.0	–	0.9	0.3	1.1	–
C _{12:0} 3-OH	–	–	–	2.7	1.5	0.5
C _{12:1} 3-OH	2.9	–	–	–	–	4.7
C _{15:0} 2-OH	–	0.9	–	–	–	–
C _{16:0} 2-OH	0.9	15.2	–	–	–	–
C _{17:0} 2-OH	–	0.6	–	–	–	–
C _{18:1} 2-OH	–	5.0	–	–	–	–
11-Methyl C _{18:1} ω7c	12.3	17.9	28.6	24.2	5.6	5.8
C _{19:0} cyclo ω8c	1.3	1.5	10.3	–	3.6	41.7
Summed features*						
1	–	–	–	0.7	–	–
3	0.3	1.7	2.2	0.4	1.3	1.2

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained iso-C_{15:1} and/or C_{13:0} 3-OH. Summed feature 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and two unidentified phospholipids. The DNA G + C content of the type strain is 63.5 mol% (determined using HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, SMK-117^T (=KCTC 22244^T =CCUG 55760^T), was isolated from sediment from a tidal flat at Saemankum (Yellow Sea, Korea).

Acknowledgements

This work was supported by the 21C Frontier Program of Microbial Genomics and Applications (grant MG05-0401-2-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea.

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